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Research Article

Drinking-Related Tetrahydroharmans Counteract the Membrane Effects of Local Anesthetic Lidocaine

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Abstract There is a general consensus in dentistry that successful local anesthesia is frequently difficult in habitual drinkers and alcoholic patients. Neuro-active tetrahydroharmans increase in human body fluids and tissues by consuming alcoholic beverages. To understand such reduced anesthetic efficacy by the drug interaction hypothesis, we studied the influences of drinking-related tetrahydroharmans on membrane fluidization as one of local anesthetic mechanisms. Liposomal membranes prepared with phosphatidylcholine and cholesterol were treated with lidocaine and different tetrahydroharmans separately and in combination, followed by measuring fluorescence polarization to determine their induced changes in membrane fluidity. In contrast to 0.1–2 mg/mL lidocaine, tetrahydroharmans decreased the fluidity of membrane preparations at $\sim 25\,\mu$g/mL with the potency being 1,2,3,4-tetrahydroharman $\gg$ 1,2,3,4-tetrahydronorharman. 1,2,3,4-Tetrahydroharman counteracted the membrane-fluidizing effects of 1 mg/mL lidocaine at physiologically relevant 0.25–2.5 ng/mL, whereas neither its 6-hydroxyl nor 7-hydroxyl metabolite did at 25–200 ng/mL. Such counteraction at a membrane lipid level may be responsible for the reduction of local anesthetic efficacy in drinkers because 1,2,3,4-tetrahydroharman increases in vivo by ingesting alcoholic beverages.

Keywords drinking; local anesthesia; reduced efficacy; lidocaine; tetrahydroharman; membrane effect

1. Introduction

Habitual alcohol consumption or chronic alcoholism induces not only pathophysiological changes such as liver disease, cardiomyopathy, coagulation alteration, gastrointestinal disturbance, and CNS dysfunction but also pharmacokinetic and pharmacodynamic changes of various drugs, especially neuro-active agents such as anesthetics and sedatives [2, 10, 22, 29]. In heavy drinkers or alcoholic patients, clinicians experience the decreased responses to or the increased dose requirements of general anesthetics in the intra-operative period [2]. Chronic alcoholism and experimental alcohol treatment make patients and animals less susceptible to general anesthetics, resulting in an increase of the effective dose for anesthesia (induction, sensory block, consciousness loss and maintenance) as reported for intravenous anesthetic barbiturates, benzodiazepines, and propofol [5, 8, 17, 24] and inhalational anesthetic isoflurane and nitrous oxide [10, 14]. Such reduction of general anesthetic efficacy has been attributed to metabolic alteration, enzymatic induction, clearance enhancement, increased liver solubility, and cross-tolerance [4, 8, 22, 29].

Local anesthesia is also affected by habitual drinking and chronic alcoholism as well as general anesthesia. Especially in dentistry, there is a general consensus that patients with heavy or long-term drinking habits face difficulty in obtaining satisfactory analgesia with local anesthetics or achieving adequate local anesthesia as described in anecdotal and case reports [16]. In animal experiments, chronic alcohol intake produces the tolerance of rats to local anesthetic lidocaine [3]. Unlike general anesthetics, however, the molecular mode of action is still arguable for the altered local anesthetic effects because pharmacokinetic mechanisms and liver uptake changes are not directly applicable to local anesthetics. It has been theorized based on the physiological changes induced by alcohol itself, including the metabolic acidosis which decreases the ability of drug molecules to diffuse through nerve sheaths and penetrate into neuronal cell membranes, and the regional vasodilation which increases blood flow and carries away drug molecules from the injection site. However, the acidosis was recently revealed not to essentially contribute to the local anesthetic failure associated with pathophysiological acidic conditions [37]. The vascular effects of alcoholic beverages are not necessarily vasodilative, but rather vasoconstrictive to produce hypertension [7]. With respect to the reduced efficacy of lidocaine, Fassoulaki et al. [3] suggested a pharmacodynamic mechanism, that is, a physicochemical change of neuronal membranes.

Besides voltage-gated sodium channels of neural and cardiac cells, local anesthetics act on membrane lipids

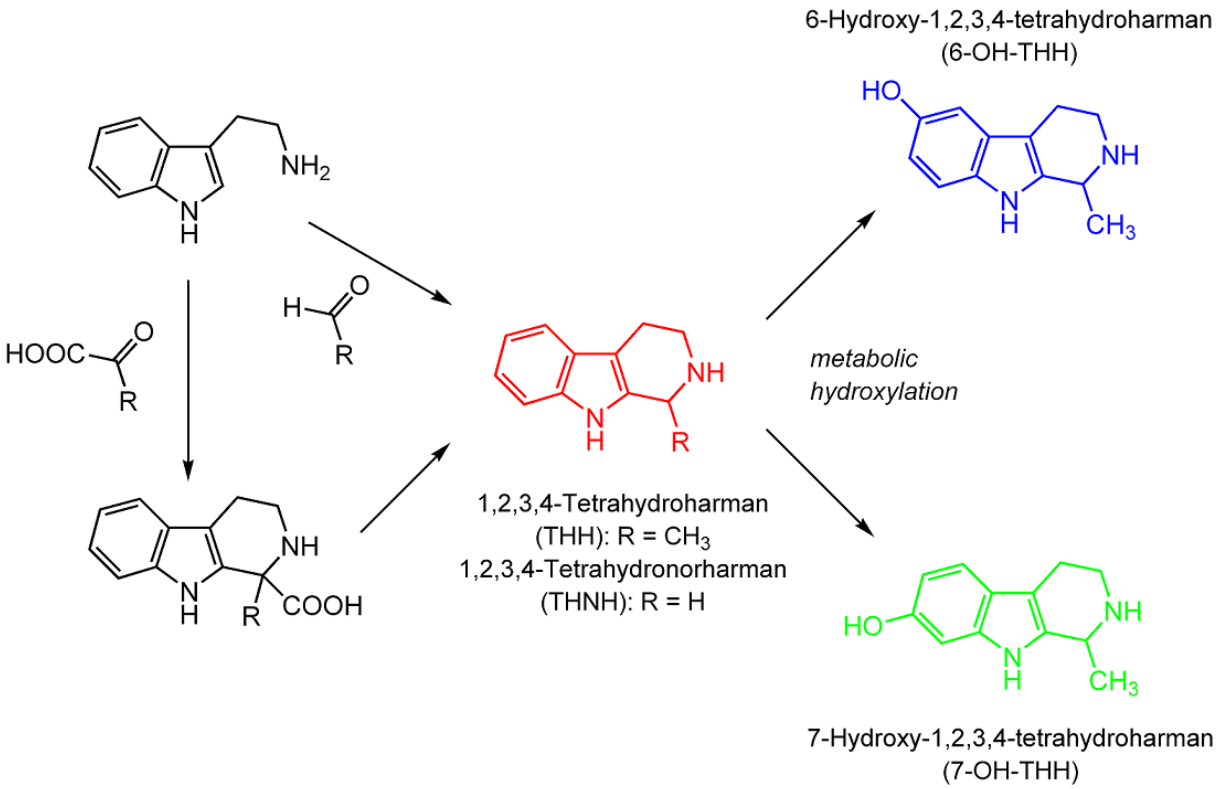

Figure 1: Structures of tetrahydroharmans.

to modify the physicochemical properties like fluidity of artificial and biological membranes, thereby directly affecting the function of biomembranes and indirectly modulating the activity of ion channels through the conformational changes of transmembrane proteins [40]. Drug-induced membrane fluidization is referred to as one of pharmacological mechanisms for local anesthetics [34].

1,2,3,4-Tetrahydroharman (THH), 1,2,3,4-tetrahydronorharman (THNH), and their structural relatives are present in the human body and also contained in various alcoholic beverages. THH and THNH are produced by the condensation reaction between tryptamine and aldehyde or α-keto acid (Figure 1). THH is metabolized to 6-hydroxy-1,2,3,4-tetrahydroharman (6-OH-THH) and 7-hydroxy-1,2,3,4-tetrahydroharman (7-OH-THH). These tetrahydroharmans significantly increase in body fluids and tissues in association with alcohol beverage consumption due to their increased exogenous supply and their promoted endogenous production [11, 36]. Certain tetrahydroharmans were recently found to affect a membrane-acting drug at a membrane lipid level [32]. In this study, we examined whether THH, THNH, 6-OH-THH, and 7-OH-THH influenced the membrane-fluidizing effects of lidocaine to get a clue to mechanistically understand the reduction of local anesthetic efficacy associated with habitual drinking.

2. Materials and methods

2.1. Chemicals

Lidocaine was purchased from Sigma-Aldrich (St. Louis, MO, USA). THH, 6-OH-THH, 7-OH-THH, and THNH were synthesized as reported previously [35]. 1,2-Dipalmitoylphosphatidylcholine (DPPC) and 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) were obtained from Avanti Polar Lipids (Alabaster, AL, USA), cholesterol from Wako Pure Chemicals (Osaka, Japan), and N-phenyl-1-naphthylamine (PNA) from Funakoshi (Tokyo, Japan). Dimethyl sulfoxide (DMSO) of spectroscopic grade (Kishida, Osaka, Japan) was used for preparing reagent solutions. All other chemicals were of the highest grade commercially available.

2.2. Membrane preparation

Liposomal membranes with the lipid bilayer structure were prepared to be unilamellar vesicles (total lipids of 0.14 mM) suspended in 10 mM Tris-HCl buffer of pH 7.4 containing 125 mM NaCl, 5 mM KCl, and 0.1 mM EDTA according to the method of Peura et al. [26]. Their lipid compositions were (1) 100 mol% DPPC (DPPC membranes) which have been most frequently used as model membranes for studying membrane-acting drugs and (2) 60 mol% POPC and 40 mol% cholesterol (POPC/cholesterol membranes) to mimic neuronal cell membranes [32].

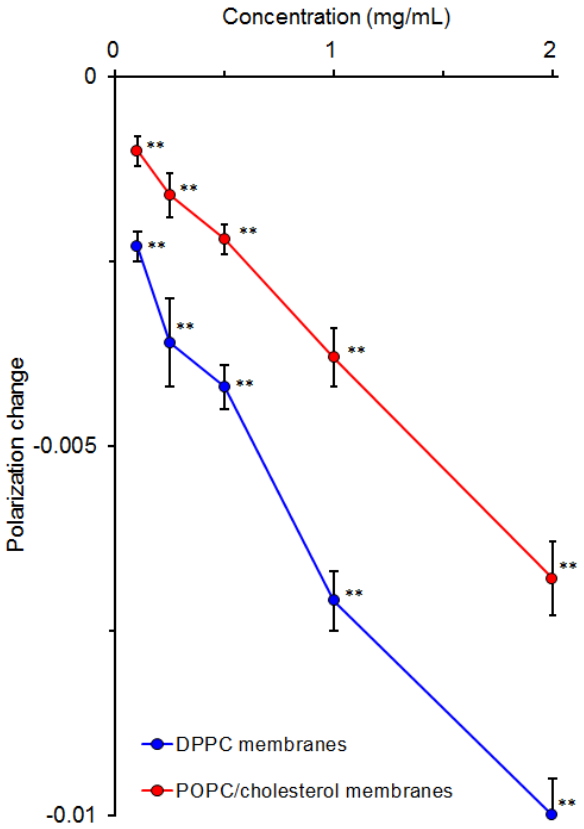

Figure 2: Effects of lidocaine on DPPC membranes and POPC/cholesterol membranes. Lidocaine was subjected to the reaction with membrane preparations at the indicated concentrations. Fluorescence polarization changes from controls were determined after labeling the membranes with PNA. Values are expressed as means ±SEM ($n = 7$). $^{**}P <$ .01 compared with controls.

2.3. Membrane effects of lidocaine and tetrahydroharmans

The solutions of lidocaine and tetrahydroharmans in DMSO were applied to the membrane preparations so that a final concentration was 0.1–2 mg/mL for lidocaine, 2 ng/mL to 25 μg/mL for THH, 20 ng/mL to 25 μg/mL for THNH, 2–200 ng/mL for 6-OH-THH, and 2–200 ng/mL for 7-OH-THH. The concentration of DMSO vehicle was adjusted to be 0.5% (v/v) of the total volume so as not to affect the fluidity of intact membranes. DMSO of the corresponding volume was added to controls. After the reaction at 37 °C for 30 min, the membranes were labeled with PNA [34], a fluorescent probe which penetrates into lipid bilayers and localizes in their hydrophobic upper regions. Since PNA is subject to the rotational restriction imparted by membrane fluidity or rigidity, drugs acting on lipid bilayers to produce more fluid membranes facilitate the probe rotation to emit the absorbed light in all directions, decreasing fluorescence polarization. Fluorescence polarization of the membrane suspensions was measured by an RF-540 spectrofluorometer (Shimadzu, Kyoto, Japan) equipped with a polarizer at 350 nm for excitation and 425 nm for emission as reported previously [33]. Polarization values were calculated by the formula $(I_{VV} - GI_{VH})/(I_{VV} + GI_{VH})$, in which I is the fluorescence intensity and the subscripts V and H refer to the vertical and horizontal orientations of the excitation and emission polarizers, respectively [38]. The grating correction factor ($G = I_{HV}/I_{HH}$) was used to correct the polarizing effects of a monochromator. Compared with controls, a decrease and an increase of polarization values mean an increase (membrane fluidization) and a decrease of membrane fluidity (membrane rigidification), respectively.

2.4. Influences of tetrahydroharmans on lidocaine membrane effects

DPPC membranes or POPC/cholesterol membranes were pretreated at 37 °C for 30 min with THH, THNH, 6-OH-THH, and 7-OH-THH of a final concentration being 0.25 ng/mL to 5 μg/mL, 5–25 μg/mL, 25–200 ng/mL, and 25–200 ng/mL, respectively. The pretreated and not-treated membranes were subjected to the reaction with lidocaine (a final concentration of 1 mg/mL) at 37 °C for 30 min, followed by measuring PNA fluorescence polarization as described above. Tetrahydroharmans and lidocaine were applied as DMSO solutions to the membrane preparations and the concentration of DMSO vehicle was 0.5% (v/v) of the total volume. Control experiments were conducted with the addition of an equivalent volume of DMSO. The inhibition (%) of lidocaine membrane effects was obtained by comparing polarization decreases induced by lidocaine with those by tetrahydroharman pretreatments.

2.5. Data analysis

Results are expressed as means ±SEM ($n = 7$). Data were statistically analyzed by a one-way analysis of variance (ANOVA) followed by a *post hoc* Fisher's protected least significant difference (PLSD) test using StatView 5.0 (SAS Institute, Cary, NC, USA). P values $< .05$ were considered significant.

3. Results

Lidocaine acted on both DPPC membranes and POPC/cholesterol membranes to increase their fluidity at 0.1–2 mg/mL as shown by PNA polarization decreases (Figure 2). The membrane-fluidizing potency varied according to lidocaine concentrations and membrane lipid compositions.

In contrast to lidocaine, THH and THNH decreased the membrane fluidity at ng/mL to low μg/mL concentrations, while THH was more effective in membrane rigidification than THNH. These tetrahydroharmans decreasingly influenced the DPPC membrane-fluidizing effects of 1 mg/mL lidocaine at $\sim$ 25 μg/mL with the potency being THH $\gg$ THNH.

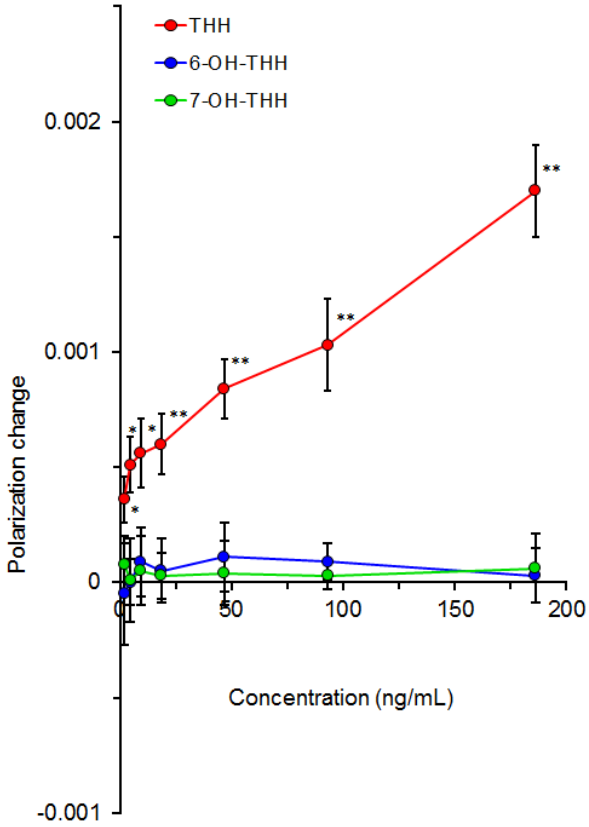

Figure 3: Effects of THH and its hydroxyl metabolites on POPC/cholesterol membranes. THH, 6-OH-THH, and 7-OH-THH were subjected to the reaction with POPC/cholesterol membranes at the indicated concentrations. Fluorescence polarization changes from controls were determined after labeling the membranes with PNA. Values are expressed as means ±SEM ($n = 7$). $^{*}P < .05$ and $^{**}P < .01$ compared with controls.

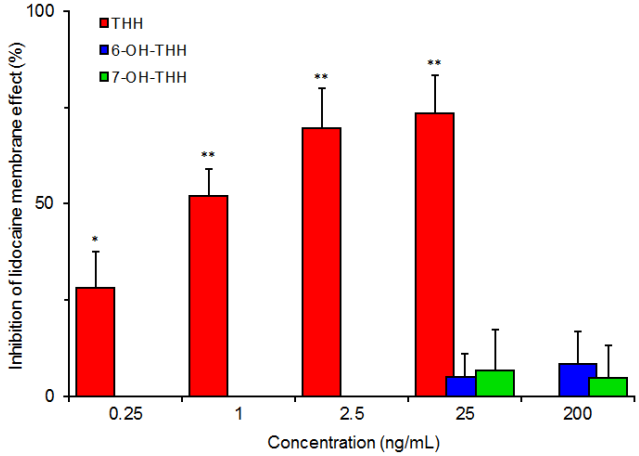

Figure 4: Counteraction of lidocaine membrane effects by THH. POPC/cholesterol membranes were pretreated with THH, 6-OH-THH, or 7-OH-THH of the indicated concentrations, followed by the reaction with 1 mg/mL lidocaine. The inhibition (%) of membrane-fluidizing effects of lidocaine was obtained by comparing PNA fluorescence polarization decreases induced by lidocaine with those by THH, 6-OH-THH, or 7-OH-THH pretreatments. Values are expressed as means ±SEM ($n = 7$). $^{*}P < .05$ and $^{**}P < .01$ compared with controls.

THH and its two hydroxyl metabolites differently acted on POPC/cholesterol membranes in a ng/mL concentration range (Figure 3). THH decreased the membrane fluidity, whereas neither 6-OH-THH nor 7-OH-THH affected it.

THH counteracted the fluidizing effects of 1 mg/mL lidocaine on POPC/cholesterol membranes at 0.25–2.5 ng/mL (Figure 4). However, 6-OH-THH and 7-OH-THH showed no significant counteraction even at 25–200 ng/mL.

4. Discussion

Our main findings are as follows: (1) lidocaine acts on lipid bilayers to increase the fluidity of DPPC membranes and POPC/cholesterol membranes at sub-mg/mL to low mg/mL concentrations; (2) tetrahydroharmans decrease the membrane fluidity to show the relative potency being THH $\gg$ THNH; and (3) the membrane-fluidizing effects of lidocaine are counteracted by 0.25–2.5 ng/mL THH, but neither by 6-OH-THH nor by 7-OH-THH.

Biomembranes play an important role as the dynamic environments to modulate the structures and functions of transmembrane or membrane-embedded proteins. Ion channels regulate the flux of ions across membrane lipid bilayers through the changes of protein conformation which is influenced not only by a binding of ligands to channels but also by an alteration of the lipid microenvironments surrounding channels. Among membrane physicochemical properties, fluidity appears to govern the dynamics of ion channels [31], and to determine the activity of sodium channels [9]. In this study, lidocaine has been revealed to increase the fluidity of both DPPC membranes and POPC/cholesterol membranes. Lidocaine induces larger fluidity changes in DPPC membranes than in POPC/cholesterol membranes, suggesting that membrane fluidization depends on the lipid composition. Its membrane effects are consistent with the previous reports of local anesthetics to fluidize artificial and synaptosomal membranes [33, 40]. The membrane-acting concentrations (0.1–2 mg/mL) of lidocaine are lower than or almost correspond to its clinically used concentrations. A relatively large volume of high concentration local anesthetics is commonly injected to ensure adequate anesthesia for surgery; that is, 20–30 mL of 20 mg/mL lidocaine are typically injected to block large nerves in humans [28]. All nociceptive C-fibers of rat sciatic nerves are blocked by the continuous superfusion with 0.33 mg/mL lidocaine [13].

Intra-neural lidocaine is related to its nerve-blocking degree and to its applied concentration. Lidocaine is likely to be concentrated in membrane lipid bilayers so that its intramembrane concentrations are higher than its concentrations in the bulk aqueous phase [28,30]. The effect to modify membrane fluidity is considered to underlie the local anesthetic action of lidocaine.

We characterized the membrane activities of tetrahydroharmans of physiologically relevant concentrations which are presumed to be sub-ng/mL to low ng/mL levels in human blood [15,18] and then investigated their influences on local anesthetic membrane effects when lidocaine and tetrahydroharmans were used concurrently. Consequently, in contrast to membrane-fluidizing lidocaine, THH has been revealed to decrease the membrane fluidity more potently than THNH, suggesting the importance of a methyl group at the 1-position. By pretreating POPC/cholesterol membranes, THH has been proved to decrease the membrane effects of lidocaine at 0.25–2.5 ng/mL, but not its hydroxyl metabolites.

THH, THNH, and harman compounds significantly increase in the human body in association with drinking behaviors and alcohol intake [11,36]. One of the general personality characteristics of habitual drinkers and chronic alcoholics is smoking. In addition to alcoholic beverages, cigarette smoke may be the source of THH and its structural relatives because they are contained in the mainstream and sidestream [27]. Their plasma concentrations are remarkably increased by alcoholic beverage ingestion and also possibly by tobacco smoking [1].

Clinicians often experience that local anesthetics are less effective on patients with a history of alcohol abuse and heavy drinking habits [6,16]. Chronic alcohol consumption produces the tolerance to lidocaine and increases its dose requirement for local anesthesia, which is not attributed to a change in lidocaine pharmacokinetics, but to a pharmacodynamic change of biomembranes [3]. Adaptation to the membrane fluidization induced by alcohol would result in a fluidity change of neuronal cell membranes or an alteration in neuronal susceptibility to alcohol membrane effects. Such resulting membrane rigidification potentially resists the membrane-fluidizing effects of local anesthetics. In alcoholic patients, erythrocyte membranes show a decrease in membrane fluidity or a pharmacodynamic tolerance to alcohol together with altering the membrane lipid composition [20,25]. Besides the membrane adaptation to alcohol, THH is able to physicochemically modify neuronal membranes.

Since tetrahydroharmans and their related compounds significantly increase in vivo by ingesting alcoholic beverages [23,36], our results suggest the possibility that THH counteracts the membrane effects of lidocaine and this counteraction may be at least partly responsible for local anesthetic failures in habitual drinkers and alcoholic patients. The counteracting activity is confined to THH, not found in 6-OH-THH and 7-OH-THH. Tetrahydroharmans are subjected to the metabolism catalyzed by cytochrome P450 enzymes in the human body, where THH is hydroxylated to form 6-OH-THH and 7-OH-THH [35]. Both hydroxyl metabolites are far less active on membrane lipid bilayers than THH because of their higher hydrophilicity. CYP2D6 and CYP2E1, which belong to the super family of cytochrome P450, are involved in the metabolism of tetrahydroharmans [12,39]. CYP2D6 and CYP2E1 are inducible by alcohol exposure and in chronic alcoholics [19,21]. If such enzymatic induction occurs, the metabolism of THH would be enhanced, with a resultant decrease of its membrane activity, thereby leading to the failure to counteract the membrane effects of lidocaine. While local anesthetics are known to be less effective on habitual drinkers and chronic alcoholics, the reduction of anesthetic efficacy may differ depending on patients. The induction of CYP isozymes converting membrane-active THH to membrane-inactive metabolites could underlie this difference.

5. Conclusion

Lidocaine increases the fluidity of lipid bilayer membranes at clinically relevant concentrations as one of local anesthetic mechanisms, whereas drinking-related THH decreases that at physiologically presumable concentrations. The membrane-fluidizing effects of lidocaine are counteracted by membrane-rigidifying THH. Such counteraction at a membrane lipid level may be related to the alteration of local anesthetic efficacy occasionally observed in habitual drinkers and alcoholic patients because THH increases in vivo by ingesting alcoholic beverages.

Conflict of interests The authors declare no conflict of interests in this study.

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